

Please replace the paragraph beginning at page 3, line 12 with the following paragraph.

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--In another related aspect, the invention features a method for treating an eye disorder in a human patient, which method involves administering to the patient a therapeutic composition that includes an ITF protein and a pharmacologically acceptable carrier. An ITF protein and biologically active fragments or variants thereof can be used for the treatment of eye disorders such as a corneal ulcer, or an ocular inflammatory disease. An ITF and biologically active fragments or variants thereof can be used to treat corneal injury or lesion associated with corneal transplantation, lens implantation and other types of eye surgery. ITF can also be used to treat traumatic physical injury to the eye. The methods of the invention also include treating eye disorders with SP or pS2 protein, e.g., a human Sp or pS2 protein (Fig. 9, SEQ ID NO:14 and Fig. 10, SEQ ID NO:16), respectively. In addition, biologically active fragments or variants of SP or pS2 can be used to treat eye disorders. Any or all of the trefoil proteins can be administered to treat an eye disorder (see Sands, Annual Rev. Physiol 58:253-73). ITF or pS2 can be administered in monomer form or can be administered in a dimer form.--

Please replace the paragraph beginning at page 4, line 28 with the following paragraph.

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--By "intestinal trefoil factor" ("ITF") is meant any protein that is substantially homologous to rat ITF (Fig. 2, SEQ ID NO.:2) or human ITF (Fig. 6, SEQ ID NO:4) and which is expressed in the large intestine, small intestine, or colon to a greater extent than it is expressed in tissues other than the small intestine, large intestine, or colon. Also included are: allelic variations; natural mutants; induced mutants; proteins encoded by DNA that hybridizes under high or low stringency conditions to ITF encoding nucleic acids retrieved from naturally occurring material; and polypeptides or proteins retrieved by antisera to ITF, especially by antisera to the active site or binding domain of ITF. The term also includes other chimeric polypeptides that include an ITF.--

Please replace the paragraph beginning at page 14, line 9 with the following paragraph.

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--Figure 3 is a depiction of the amino acid sequences of rat trefoil factor (SEQ ID NO:2), pS2 protein (SEQ ID NO:11), and pancreatic spasmolytic polypeptide (SP) (SEQ ID NO:12). The sequences are aligned to illustrate the amino acid sequence homology between the proteins. Dashes (-) indicate the insertion of spaces which optimize alignment. Bars indicate sequence identity.--

a5 Please replace the paragraph beginning at page 14, line 17 with the following paragraph.

--Figure 5 is a depiction of the proposed disulfide bond structure of rat ITF (residues 23-81 of SEQ ID NO:2).--

a6 Please replace the paragraph beginning at page 14, line 19 with the following paragraph.

--Figure 6 is a depiction of the nucleotide sequence of the human ITF cDNA (SEQ ID NO:3) and the corresponding deduced amino acid sequence (SEQ ID NO:4) --

a7 Please replace the paragraph beginning at page 14, line 28 with the following paragraph.

--Figure 9 is a depiction of the nucleotide sequence of the mature human spasmodic cDNA and the corresponding deduced amino acid sequence (SEQ ID NO:13 and 14, respectively).--

a8 Please replace the paragraph beginning at page 15, line 1 with the following paragraph.

--Figure 10 is a depiction of the nucleotide sequence of the human pS2 cDNA and the corresponding deduced amino acid sequence (SEQ ID NO:15 and 16, respectively).--

Please replace the paragraph beginning at page 25, line 21 with the following paragraph.

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--It is possible to isolate part of the hITF gene directly from the packaged library or cDNA. To isolate a portion of hITF directly from the packaged library, a pair of oligonucleotide primers and Taq polymerase are used to amplify the DNA corresponding to the hITF gene. The primers used would be approximately 15-20 nucleotides long and correspond in sequence to the 5'-most and 3'-most portions of the rITF coding sequence. Friedman et al. (in PCR Protocols: A Guide to Methods and Applications, Innis et al., Eds., Academic Press, San Diego) describe a procedure for such amplification. Briefly, phage particles are disrupted by heating; Taq polymerase, primers (300 pmol of each), dNTPs, and Taq polymerase buffer are added; and the mixture is thermally cycled to amplify DNA. The amplified DNA is isolated by agarose gel electrophoresis. The ends of the fragment are prepared for ligation into an appropriate vector by making them flush with T4 polymerase and, if desired, adding linkers. Alternatively, a restriction site may be engineered into the fragment by using primers which have sequence added to their 5' ends which sequence will generate an appropriate sticky end when digested. For example the sequence: 5'-GGGCGGCCGC-3' (SEQ ID NO:5) can be added to the 5' end of each primer. This sequence includes the NotI restriction site flanked at the 5' end by the sequence: GG. The additional nucleotides prevent the 5' ends from denaturing and interfering with

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subsequent restriction digestion with NotI. The gel purified DNA of the appropriate size is next cloned into a cloning vector for sequencing and restriction mapping. This clone will not have the entire hITF sequence, rather it will be a combination of hITF (the region between the sequences corresponding to the primers) and rITF (the 5' and 3' ends which correspond to the primer sequences). However, this DNA can be used to generate a labelled probe (produced by nick translation or random primer labelling) which, since it is the correct hITF sequence, can be used in a high stringency screening of the library from which the cDNA was originally isolated. In an alternative approach, cDNA can be used in the above procedure instead of a packaged library. This eliminates the steps of modifying the cDNA for insertion into a vector as well as cDNA packaging and library amplification. Ausubel et al. supra provides a protocol for amplification of a particular DNA fragment directly from cDNA and a protocol for amplification from poly(A)⁺ RNA.--

Please replace the paragraph beginning at page 27, line 16 with the following paragraph.

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--A human intestinal cDNA library was obtained from Clontech (Palo Alto, CA). Alternatively, a human intestinal cDNA library may be produced from mucosal scrapings as described above. Four oligonucleotide probes were selected for screening the library cDNA. Two of the probes correspond to sequences within the region of rITF encoding the trefoil and are referred to as internal probes (5'-GTACATTCTGTCTCTTGCAGA-3' (SEQ ID NO:6) and 5'-TAACCCTGCTGCTGCTGGTCCTGG-3' (SEQ ID NO:7)). The other two probes recognize sequences within rITF but outside of the trefoil encoding region and are referred to as external probes (5'-GTTTGCGTGCTGCCATGGAGA-3' (SEQ ID NO:8) and 5'-CCGCAATTAGAACAGCCTTGT-3' (SEQ ID NO:9)). These probes were tested for their utility by using them to screen the rat intestinal cDNA library described above. Each of the four probes could be used to identify a clone harboring all or part of the rITF gene. This result indicates that these probes may be used to screen the human intestinal library for the presence of hITF.--

Please replace the paragraph beginning at page 28, line 28 with the following paragraph.

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--Figure 6 shows the nucleic acid sequence information from the human ITF cDNA clone (SEQ ID NO:3) ~~that was deposited with the ATCC on _____, 1998~~, along with the deduced amino

Ant acid sequence in one-letter code (SEQ ID NO.:4). This clone was obtained in the experiment described above.--

Please replace the paragraph beginning at page 29, line 30 with the following paragraph.

a¹² --Anti-ITF monoclonal antibodies can be raised against synthetic peptides whose sequences are based on the deduced amino acid sequence of cloned hITF (SEQ ID NO.:4). Most commonly the peptide is based on the amino-or carboxy-terminal 10-20 amino acids of the protein of interest (here, hITF). The peptide is usually chemically cross-linked to a carrier molecule such as bovine serum albumin or keyhole limpet hemocyanin. The peptide is selected with the goal of generating antibodies which will cross-react with the native hITF. Accordingly, the peptide should correspond to an antigenic region of the peptide of interest. This is accomplished by choosing a region of the protein which is (1) surface exposed, e.g., a hydrophobic region or (2) relatively flexible, e.g., a loop region or a β -turn region. In any case, if the peptide is to be coupled to a carrier, it must have an amino acid with a side chain capable of participating in the coupling reaction. See Hopp et al. (1983, Mol. Immunol. 20:483; 1982, J. Mol. Biol. 157:105) for a discussion of the issues involved in the selection of antigenic peptides. A second consideration is the presence of a protein homologous to hITF in the animal to be immunized. If such a protein exists, it is important to select a region of hITF which is not highly homologous to that homolog.--

Please replace the paragraph beginning at page 32, line 5 with the following paragraph.

a¹³ --The polymerase chain reaction (PCR) was used to confirm the targeted mutation as follows. A 200 bp region of DNA was amplified using primers spanning exon 2 of ITF (5'-GCAGTGTAACAACCGTGGTTGCTGC-3'(SEQ ID NO.:10) and 5'-TGACCCTGTGTCATCACCTGGC-3'(SEQ ID NO.:17)); and a 400 bp region of the neo gene was amplified with a second set of primers (5'-CGGCTGCTCTGATGGCCGCC-3'(SEQ ID NO.:18) and 5'-GCCGGCCACAGTCGATGAATC-3'(SEQ ID NO.:19)). The DNA template for the PCR reaction was obtained from tail tissue. Approximately 0.5 cm of the tail was cut off each animal, and the samples were digested with proteinase-K (200 μ l at 0.5 mg/ml in 50 mM Tris-HCl pH 8.0 and 0.5% Triton X-100; Sigma, St. Louis, MO) at 55°C overnight. One μ l of this mixture was added directly to a 25 μ l PCR reaction (per Stratagene, Menasha, WI). The reaction was begun with a "hot start" (incubation at 96°C for 10 minutes), and the following

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cycle was repeated 30 times: 72°C for 120 seconds (hybridization and elongation) and 96°C for 30 seconds (denaturation). Ten µl of each reaction mixture was electrophoresed on a 2% agarose gel. Wild type animals were identified by the presence of a 200 bp fragment, corresponding to an intact ITF gene, heterozygous animals were identified by the presence of this band and, in addition, a 400 bp fragment produced by amplification of the neo gene, and ITF-deficient (knock out) animals were identified by the presence of only the fragment corresponding to the neo gene.--

Please replace the paragraph beginning at page 33, line 5 with the following paragraph.

all
--Although expression of ITF is abolished in the mutant mice, expression of other trefoil genes is preserved. Northern blot analysis was performed using cDNA probes for ITF (Suemori et al., 1991, Proc. Natl. Acad. Sci. USA 88:11017), SP (Jeffrey et al., 1994 Gastroenterology 106:336), and, as a positive control, glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The nucleic acid probe for murine pS2 was made by reverse transcription-polymerase chain reaction (RT-PCR) using the oligonucleotide pairs: 5'-GAGAGGTTGCTGTTTTGATGACA-3' (SEQ ID NO.:20) and 5'-GCCAAGTCTTGATGTAGCCAGTT-3' (SEQ ID NO.:21), which were synthesized based on the published mouse pS2 cDNA sequence (GenBank Accession Number: Z21858). The GeneAmp RNA PCR Kit (Perkin Elmer) was used according to the manufacturer's instructions, as was the pCRJII (Invitrogen) cloning vector. RNA was extracted from the following tissues from both wild type and ITF-deficient (knock out) mice: stomach, duodenum, terminal ileum, right colon, appendix, transverse colon, left colon, and rectum. Fifteen µg of total RNA from each sample were electrophoresed on a 1% agarose gel, and transferred to nitrocellulose paper. Following hybridization, washing, and autoradiography, wild type mice exhibited a pattern of tissue expression considered normal: ITF was expressed in the small intestine and colon, which is the same expression pattern seen for ITF in the rat and human. The analysis of mutant mice confirmed the lack of ITF expression in the gastrointestinal tract. In contrast, the expression of the other trefoil proteins, SP and pS2, are unaltered in the gastrointestinal tract of mutant mice. SP was expressed in the stomach and, at lower levels, in the duodenum of both wild type and mutant mice. Similarly, pS2 was expressed in the stomach of both wild type and ITF-deficient mice.--

Please insert the paper copy of the Sequence Listing (attached) following the drawings.